IV. NOTE ON THE EFFECT OF SODIUM TRICHLOROACETATE ON THE REDUCTION OF COPPER SOLUTIONS BY GLUCOSE.

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As a preliminary to some investigations on glucose, it was thought necessary to test the effect, if any, of protein precipitants on the accuracy of glucose estimations. The quantitative methods examined were the Bertrand method and the micro-method of Schaffer and Hartmann [1921]. It was observed that when trichloroacetic acid was used to precipitate the proteins and the glucose in the filtrate determined by either of the above methods, the percentage of glucose found was always very much lower than that of a control, done on a glucose solution not treated with trichloroacetic acid. This discrepancy was traced to the presence of sodium trichloroacetate which can inhibit the reduction provided the salt be present in sufficiently great concentration.

The experiments quoted below were done on the Schaffer-Hartmann micro-method to try and find the cause of this result.

The results of Exp. 1 show the extent of this inhibiting effect.

Exp. 1. 3 cc. of a 0.05 % glucose solution were used. Water was added to bring the total volume up to 5 cc.

<i>N</i> /10 trichloroacetate added	Cuprous oxide found
cc.	mg.
0	2.39
1.0	0.99
1.5	0.26
2.0	0.23

5 cc. of the Schaffer copper solution, heated with 5 cc. of water, required 19.4 cc. *N*/200 thiosulphate. The same volume of thiosulphate was required by another control—not heated—but with 5 cc. of *N*/10 trichloroacetate in place of the water.

The presence of the trichloroacetate in a concentration of 0.02 *N* so inhibits the reduction that there is no visible formation of cuprous oxide, although the titration indicates a very small reduction of the copper. The cupric copper of the control is unaffected by the presence of trichloroacetate.

Polarimetric evidence of a reaction between the glucose and trichloroacetic acid is entirely negative. Moreover as it was noticed that the appearance of the cuprous oxide, if formed, is retarded when the trichloroacetate is present, experiments were performed to see if any evidence could be obtained, indicating a reaction between the copper and the trichloroacetate.

Exp. 2. Four samples of the following mixture were made up: 5 cc. of Schaffer copper solution + 1.5 cc. of sodium trichloroacetate *N*/10 + 0.5 cc. of water.

This mixture was heated for various periods on the water bath prior to adding the glucose. After the addition of the 3 cc. of glucose solution, heating was continued for another 15 minutes.

	Cuprous oxide found mg.
1. Heated for 5 minutes before addition of glucose	1.49
2. " 10 " " "	2.89
3. " 15 " " "	3.21
4. Not heated	0.22

That it is the undecomposed trichloroacetate which is active is shown in *Exp. 3.* The reduction can be stopped at any stage after it has started, by adding the trichloroacetate. The cuprous oxide already formed is not destroyed.

Exp. 3. 3 cc. glucose solution + 2 cc. water heated with 5 cc. of the Schaffer copper solution. At various stages of the reduction 0.5 cc. of a 0.5 *N* solution of trichloroacetate was added.

Time of adding trichloroacetate	Cuprous oxide found mg.
After 5 minutes reduction	2.54
" 10 " "	3.08
" 15 " "	3.37
No trichloroacetate added	3.41

These results seem to indicate that there occurs a reaction between the copper of the Schaffer solution and the sodium trichloroacetate by which the copper passes into a non-reducible complex. This organo-copper compound decomposes gradually on heating—like sodium trichloroacetate itself; but during the heating the glucose will be destroyed by the sodium carbonate of the copper solution and no reduction—or very little—will result, even after the copper has reverted to its reducible condition.

Doughty [1919] and Doughty and Freeman [1922] have obtained evidence of an organo-copper complex of trichloroacetic acid but its behaviour on heating with glucose in alkaline solution has not been investigated.

Since the above experiments were made, the author has found a reference to this effect of trichloroacetic acid, in a paper by Denigès [1922] in which he states that this acid "apporte des perturbations profondes sur le pouvoir réducteur des sucres." Beyond this observation no investigations seem to have been made with a view to elucidating the mechanism of the effect.

CONCLUSIONS.

The use of trichloroacetic acid as a protein precipitant prior to glucose estimations by any of the copper reduction methods is to be avoided. There is experimental evidence of the formation of an unstable organo-copper compound of trichloroacetic acid in which the copper is non-reducible.

REFERENCES.

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